

Construct 1: J23100 and T7 expression of full synthesis product

Construct 2: Antibody insertion into modified synthesis construct without MCherry

Construct 3: OsmY insertion into psb1C3

Construct 4: Full synthesis without Mcherry and with T7/J23100

MAY

5/8/2014

- Transformed J06505 (MCherry) with DiRita Lab DH5a CC E. Coli. We transformed them onto Cm Plates (J06505 CMr).

- Started an overnight culture of Bardwell Lab strain harboring pOsmY (Cut sites NdeI (n terminus) and BamHI (c terminus)).

- Transformed part T7 promoter + B0034 (K525998). Plate: 2013 Kit Plate 1 well 3M. Resistance: Cm

5/9/2014

- J06505 (MCherry) transformation was successful (harbored ~10 colonies), colonies were plates onto new plate containing all 10 for further screening

- Mini-prep of Bardwell Lab's OsmY-plasmid containing strain

5/13/2014

- OsmY Plasmid digest with NdeI and BamHI

- Ran a gel to confirm band size

- Transformed part T7 promoter + B0034 (K525998) from 2013 Kit Plate 1 well 3M, and plated on to a chloramphenicol resistant plate

5/14/2014

- Started cultures of T7 + B0034 from transformed plates (and made replica plate) using NEB 10 beta and DH5 alpha comp cells

- Transformed part J18918 Plate: 2013 Kit Plate 5 well 16I (Amp Resistance)

5/15/2014

- Mini-prepped all T7 + B0034 cultures

- Looked at the J18918 plate at 11am, but it didn't look promising so we left it in the incubator until 12pm, then put it in the fridge to look at in the evening and possibly redo the transformation

- Mini-prepped all T7 + B0034 cultures, and put them in the freezer

5/28/2014

- Started overnight cultures of J06505 (MCherry) and J23100 (Constitutive promoter)

- 1 hour Digest of T7+RBS with S/P

5/29/2014

- Mini-prepped MCherry and J23100

- Digested MCherry with X/P, and then gel extracted MCherry
- Digested J23100 with S/P, gel purified, and ran out the product on a gel to confirm that the vector was linearized
- The concentrations of DNA was too low for the ligation, so we will repeat this

6/4/2014

- Transformed J23100 + MCherry
- Transformed T7+B0034 + MCherry